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Mucosal immunization with a measles virus CTL epitope encapsulated in biodegradable PLG microparticles

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Abstract

The immunogenicity of a cytotoxic T cell epitope (CTL) representing residues 52–60 from measles virus (MV) nucleoprotein, encapsulated in poly(lactide-co-glycolide) (PLG) microparticles was evaluated after mucosal immunization. After intranasal administration of the encapsulated CTL epitope linked at the carboxyl terminus of two copies of a T-helper epitope (TT-NP6), peptide-specific and MV-specific CTL responses were detected in splenocytes. However, these responses were lower than the responses observed when the TT-NP6 peptide was administered intranasally in saline or using CTB as an adjuvant. Intranasal coadministration of the encapsulated TT-NP6 peptide with CTB did not result in any significant potentiation of the CTL responses. The effectiveness of biodegradable PLG microparticles for mucosal delivery of CTL epitopes, combined with their excellent tissue compatibility and biodegradability suggests that they represent a valuable delivery system for synthetic immunogens. However, further work is needed to define the requirements for effective absorption by the nasal epithelium.

Keywords: Cytotoxic T cell epitope; Intranasal immunization; Measles virus; Poly(lactide-co-glycolide) microparticle

1. Introduction

Although most human pathogens gain entrance via the mucosal surfaces, the majority of the currently available vaccines have been developed for systemic immunization. To a large extent this reflects the relative difficulty in achieving effective mucosal immunization. Numerous studies in animals and humans have provided convincing evidence that protection against a variety of viral and bacterial mucosal pathogens can be obtained by oral or intranasal vaccination (Moldoveanu et al., 1993; Tamura et al., 1992). This has resulted in an intense search for safe and immunogenic mucosal vaccines. However, a major problem has been the poor bioavailability of antigens delivered mucosally, due to proteolytic degradation. For this reason much attention has been directed towards the development

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of delivery systems for mucosal vaccination and PLG microparticles have been considered a candidate for mucosal vaccine delivery. Unlike soluble antigen, mucosally administered antigen entrapped in PLG microparticles can induce good local and systemic antibody responses (Eldridge et al., 1991; Maloy et al., 1994). However, the ability of this delivery system to induce CTL responses has not been extensively studied. Intraperitoneal delivery of PLG encapsulated MV CTL epitopes induces long term peptide- and MV-specific CTL responses (Partidos et al., 1996a). In this report, we describe the potential of PLG microparticles for the administration of peptides representing MV CTL epitopes via the intranasal route.

2. Materials and methods

The NP6 CTL epitope from MV nucleoprotein (NP), representing residues 52–60 (LDRLVRLIG) (NP6) (Beanverger et al., 1994) was co-linearly synthesized using Fmoc chemistry at the carboxyl terminus of two copies (TT-NP6) of a T-helper epitope (T) representing residues 288–302 from the fusion protein of MV (Partidos and Steward, 1990; Partidos et al., 1996b). The chimeric peptide was encapsulated in PLG microparticles by a solvent extraction method as described previously (Jones et al., 1995). The mean diameter of the microparticles was 1.88 μm and the efficiency of encapsulation was 30%.

The immunogenicity of TT-NP6 peptide was tested after intranasal administration of groups of three CBA mice (6–8 weeks old) with 50 μg/dose of peptide either encapsulated (group A), free in saline (group B) or coadministered with cholera toxin B subunit (CTB) (Sigma, 10 μg/dose) free peptide in saline (group C) or encapsulated peptide (group D) in 30 μl volume of phosphate buffer saline on 3 consecutive days. Mice were boosted 3 weeks later with a single dose of 50 μg/dose of peptide encapsulated (group A), free in saline (group B) or coadministered with CTB (10 μg/dose) free peptide in saline (group C) or encapsulated peptide (group D).

Two weeks after intranasal administration, immune splenocytes were harvested and restimulated in vitro with the NP6 peptide for 7 days. On day 3, 10% (v/v) rat Con A supernatant (as a source of IL-2) was added to the cultures. CTL activity was assessed using the ^{51}Cr release assay as described previously (Partidos et al., 1996b).

3. Results and discussion

The potential of the intranasal route for mucosal immunization was tested with the encapsulated TT-NP6 chimeric peptide. As shown in Fig. 1, effector splenocytes from mice immunized with the TT-NP6 peptide with CTB as an adjuvant or free in saline could lyse L929 (panel a) or NS20Y (panel b) (Rager-Zisman et al., 1984; Gopas et al., 1992) target cells pulsed with 1 μM of NP6 peptide or persistently infected with MV (panel c) more effectively than effector cells from mice immunized with encapsulated TT-NP6 peptide. No significant lysis was observed in non-pulsed target cells. Furthermore, when mice were immunized intranasally with encapsulated TT-NP6 peptide coadministered with CTB, no significant potentiation of CTL lysis was observed to either L929 (Fig. 2a), NS20Y (Fig. 2b) target cells pulsed with 1 μM of NP6 peptide or to NS20Y/MS persistently infected with MV target cells (Fig. 2b).

For the development of synthetic peptide immunogens with the potential to induce CTL responses, the peptides must have the ability to insert into the cell membrane of antigen presenting cells (APCs) and facilitate processing via the class I pathway. The finding that PLG microparticles can act as a delivery system for CTL epitopes suggests that after being taken up by APCs they can escape into the cytoplasm where the released CTL epitope could then be transported to the endoplasmic reticulum by a cytoplasmic peptide transporter (Yewdell and Bennick, 1990; Discoll and Finley, 1992). Thus, the CTL epitope gains access to the pathway for class I presentation. Studies by Eldridge et al. (1991) have suggested that the predominant mechanism of immune enhancement by microparticles is the direct intracellular delivery to accessory cells of high concentrations of antigen incorporated within the copolymer matrix. Indeed, microparticles can be rapidly phagocytosed by macrophages (Tabata and Ikada, 1990) which can serve as APCs for the generation of CTLs (Debrick et al., 1991).

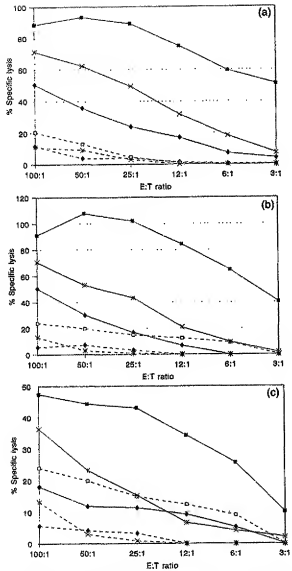

Fig. 1. CTL activity induced after intranasal priming of groups of CBA mice with the TT-NP6 peptide administered free in saline (⋆), encapsulated in PLG microparticles (◆) or coadministered with CTB (■). After in vitro restimulation of splenocytes with NP6 peptide, effector cells were cocultured with L929 (*a*), NS20Y (*b*) target cells pulsed (————) or non-pulsed (– – –) with 1 μM of NP6 peptide or the persistently infected MV NS20Y/MS cell line (————) (*c*) at the indicated E:T ratio. Spontaneous release was 12% for L929 cells and 24% for NS20Y and NS20Y/MS cells of the total release by detergent in all assays. Data represent the mean of triplicates for each E:T ratio after pooling the spleen cells from groups of three mice. The SD of triplicate wells was consistently less than 10% of the mean.

The effectiveness of PLG microparticles as a delivery system is further highlighted by their ability to induce CTL responses after intranasal immunization. Successful induction of systemic and mucosal antibody responses after mucosal administration of PLG microparticles has been reported for several microencapsulated antigens (Moldoveanu et al., 1993; Eldridge et al., 1991) and in recent reports PLG microparticles have been shown to prime for ovalbumin (OVA)-specific CTL responses after parenteral and oral immunization (Maloy et al., 1994) and HIV-specific CTL responses after intranasal pri-

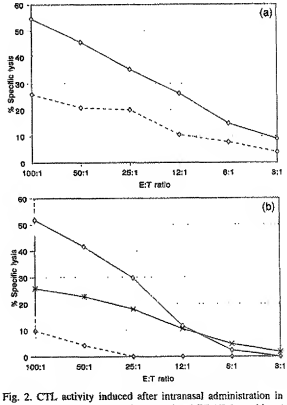

Fig. 2. CTL activity induced after intranasal administration in a group of three CBA mice of encapsulated TT-NP6 peptide with CTB. After in vitro restimulation of splenocytes with NP6 peptide, effector cells were cocultured with L929 (*a*), NS20Y (*b*) target cells pulsed (————) or non-pulsed (– – –) with 1 μM of NP6 peptide or with the persistently infected MV NS20Y/MS cell line (*b*) (⋆ ———— ⋆) at the indicated E:T ratio. Spontaneous release was 10.58% for L929 cells and 28% for NS20Y and NS20/MS cells of the total release by detergent in all assays. Data represent the mean of triplicates for each E:T ratio after pooling the spleen cells from a group of three mice. The SD of triplicate wells was consistently less than 10% of the mean.

minig with encapsulated gp120 protein (Moore et al., 1995). The results presented here are consistent with these findings and demonstrate for the first time the potential of PLG microparticles for the delivery of synthetic peptides representing CTL epitopes via the intranasal route. However, the responses with the encapsulated peptide were lower than the responses observed after intranasal administration with free peptide in saline or free peptide coadministered with CTB. This suggests that the microparticles might not be absorbed efficiently by the nasal epithelium. It has been suggested that soluble antigens may easily penetrate the whole nasal epithelium as compared to particulate antigens which may be removed quickly from the nasal mucosa by the mucociliary system (Kuper et al., 1992). Moreover, although CTB has been suggested to exert its adjuvanticity by enhancing the transepithelial influx of the vaccine into the nasal mucosa where the immunocompetent cells are located (Gizurarson et al., 1992), its coadministration with the encapsulated peptide did not result in any significant potentiation of CTL responses. Thus. although it appears that biodegradable PLG microparticles can act as an effective delivery system for mucosal immunization of synthetic peptides representing viral CTL epitopes, further work is needed to define the requirements for more effective absorption by the nasal epithelium.

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